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| (54) Title: ATTACHMENT ENHANCED 293 CELLS (57) Abstract Attachment enhanced human embryonic kidney cells, 293, are provided. These cells have been modified to contain a selected mammalian scavenger gene, which has been found to improve the ability of these cells to attach in culture. The improved cells of the invention are useful in assays in which the unmodified 293 cells could be used. | | |

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ATTACHMENT ENHANCED 293 CELLS

Field of the Invention

This invention relates generally to cell lines used in the recombinant
5 production, screening or measurement of protein or protein interactions *in vitro*.

Background of the Invention

The primary human embryonic kidney (HEK) 293 cell line is a permanent
line of cells transformed by sheared human adenovirus type 5 (Ad 5) DNA. The
10 cells are particularly sensitive to human adenovirus, are highly permissive for
adenovirus DNA, and contain and express the transforming genes of Ad5. This is a
hypotriploid human cell line. See, F. Graham *et al.*, J. Gen. Virol., 36:59-72 (1977);
T. Harrison *et al.*, Virology, 77:319-329 (1977).

This cell line, which is readily available from commercial sources, such as
15 the American Type Culture Collection, is used extensively in *in vitro* assays, and for
the production of recombinant proteins and viruses. However, in washing steps
which are conventionally and repeatedly employed in such *in vitro* assays and other
manipulations of these cells, the cells readily detach or are washed away from the
plates or dishes in which the studies are performed. This problem typically results in
20 inaccurate, unreliably low measurement or collection of the protein, peptide or
interaction to which the assay is directed.

There remains a need in the art for a cell substrate useful in *in vitro*
manipulations in genetic engineering, which permits the measurement of accurate
results.

25

Brief Summary of the Invention

In one aspect, the invention provides improved HEK 293 cells, which cells
are 293 cells which have been transfected with a mammalian macrophage scavenger
receptor gene. Preferably, this gene is the human Type I or II macrophage scavenger
30 receptor gene [SEQ ID NOS: 1 or 3].

In another aspect, the invention provides a method of enhancing the ability of
HEK 293 cells to attach in tissue culture. This method involves the steps of

transfecting 293 cells with a selected mammalian macrophage scavenger receptor gene.

In yet another aspect, the invention provides a method of screening compounds for biological activity which involves screening the improved 293 cells of the invention. In this method, the improved 293 cells have been further transfected with a selected gene and are then screened for expression of the selected gene. The cells expressing the selected genes are incubated in the presence of a compound of unknown biological activity, and then screened for the ability of the compound to affect the expressed gene product or its function.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 provides the nucleic acid [SEQ ID NO:1] and amino acid [SEQ ID NO:2] sequences of the human macrophage scavenger receptor type I.

Fig. 2 provides the nucleic acid [SEQ ID NO:3] and amino acid [SEQ ID NO:4] sequences of the human macrophage scavenger receptor type II.

Detailed Description of the Invention

The present invention provides an improved human embryonic kidney cell line, 293. The inventors have surprisingly found that human embryonic kidney (HEK) 293 cells transfected with a mammalian macrophage scavenger receptor gene demonstrate an enhanced ability to attach to a solid support as compared to conventional, unmodified 293 cells. In contrast to unmodified 293 cells, the improved 293 cells of the invention are not as readily washed away as unmodified 293 cells under the normal conditions of biological assays. Thus, the improved 293 cells of the invention are particularly well suited for use in *in vitro* studies and other applications for which unmodified 293 cells may be used.

As used herein "solid support" is any surface used for culturing, for *in vitro* assays, and the like. For example, a typical solid support is a plastic tissue culture plate, or a multi-well plate, hollow fibers, a test tube, conventionally employed

plastic beads, glass beads, etc. Other solid supports are well known to those of skill in the art.

By "enhanced ability to attach" is meant that the transfected cells of this invention attach to the solid support with sufficient avidity to resist detachment which normally occurs with untransfected 293 cells caused by assay washing steps with buffer or growth medium. More specifically, the transfected cells of this invention because of the characteristic of enhanced attachment provide results of, for example, five times the cell number remaining after two washes as compared to the number of cells remaining following two washes of untransfected cells.

10 The human embryonic kidney cell line, 293, is readily available from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, U.S.A., under accession number ATCC CRL 1573. Also encompassed by this invention are progeny and derivatives of this cell line, which may be prepared using conventional techniques. See, Sambrook, Molecular Cloning: A Laboratory
15 Manual, 2d ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

According to this invention, these cells are modified by transfection with a selected mammalian macrophage scavenger receptor (MSR) gene. Currently, in a preferred embodiment, this gene is selected from a human MSR Type I or Type II gene, and most preferably, the gene is characterized by the sequence provided in
20 GenBank, under accession number D90187 (MSR Type I) or D90188 (MSR Type II). The sequences [SEQ ID NO: 1 and 2] of MSR Type I are provided in Fig. 1. The sequences [SEQ ID NO: 3 and 4] of MSR Type II are provided in Fig. 2. Both of these genes were obtained from the human monocytic cell line THR-1 following four days of phorbol ester treatment. These two gene sequences are differential
25 splice variants of a single human gene, and are described in more detail in A. Matsumoto *et al.*, Proc. Natl. Acad. Sci. USA, 87:9133-9137 (1990), incorporated by reference herein.

It is anticipated that non-human homologs of MSR I or MSR II will be similarly useful in preparing the improved 293 cells according to the invention.
30 Particularly desirable are the bovine [T. Kodama *et al.*, Proc. Natl. Acad. Sci. USA, 85:9238-9242 (1988)], murine [M. Freeman *et al.*, Proc. Natl. Acad. Sci. USA,

87:8810-8814 (1990)] and rabbit [P. E. Bickel and M. W. Freeman, J. Clin. Invest.,
90:1450-1457 (1992)] homologs, each of which is at least 60-80% homologous with
the human MSR genes. It is further anticipated that other human scavenger receptor
genes, particularly other genes which are produced recombinantly or are
5 differentially selective for oxidized or acetylation-modified low density lipoprotein
(LDL) species or another desired lipoprotein species, will be similarly useful.

One of these genes, preferably a human MSR gene, is selected and cloned
into an appropriate vector for use in transfecting the 293 cells. Generally, a suitable
expression vector is one which contains control or regulatory sequences operably
10 linked with the nucleic acid sequences of the gene. These regulatory sequences are
capable of directing the expression of the gene product in the 293 cells. Suitable
vectors and regulatory sequences are well known to those of skill in the art and this
invention is not limited by the selection thereof.

For example, suitable vectors may be, or contain components from, viral
15 vectors selected from simian virus SV40, retroviruses, bovine papilloma virus,
vaccinia virus, and adenovirus, or commonly used bacterial vectors or commonly
used mammalian expression vectors or integrative vectors which lead to a stable
expression cell line. The vector used in the examples below is pCDN [N. Aiyar *et*
al., Mol. Cell. Biochem., 131:75-96 (1994)], which contains the promoter from
20 cytomegalovirus, followed by a polycloning site and a polyadenylation site, the
SV40 early enhancer, the human gene for dihydrofolate reductase, and a gene
conferring resistance to neomycin.

Methods for introduction of a vector containing an MSR gene into
mammalian cells are well known. Examples of suitable methods include, without
25 limitation, dextran-mediated transfection, calcium phosphate precipitation,
polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of
the polynucleotide(s) in liposomes, and direct microinjection of the DNA into
nuclei.

Sequences which contain selectable markers may also be transfected into the
30 cell line. These markers may be contained on the vector containing the MSR gene,
or may be separately transfected using conventional techniques, such as those

described herein. Selectable markers for mammalian cells are known in the art, and include for example, thymidine kinase, dihydrofolate reductase (together with methotrexate as a DHFR amplifier), aminoglycoside phosphotransferase, hydromycin B phosphotransferase, asparagine synthetase, adenosine deaminase, 5 metallothionien, and antibiotic resistant genes such as neomycin. Other markers may be readily selected by one of skill in the art, as desired.

As described in more detail below, if the MSR transfected cell is desired for use in a screening assay, the cell may also be transfected with other genes. The additional gene(s) may, for example, encode a protein which will be screened for 10 biological activity or for interaction with the MSR or another transfected gene.

Following transfection with the selected MSR gene (and optionally, any other gene), the cells are incubated in a suitable selection medium, e.g., Eagles MEM, Dulbecco's MEM or the like.

Once modified to contain the MSR gene, or another suitable gene, according 15 to the methods described above, the improved 293 cells are particularly well suited for use in any assay in which an unmodified 293 cell may be used. However the use of the improved 293 cells of the invention will result in superior attachment, and thus, more accurate test results.

An exemplary use of the improved 293 cells of the invention includes the use 20 of these cells in a method of screening compounds for biological activity. This method involves the use of the attachment enhanced 293 cells of the invention which have been further transfected with a selected gene sequence. These cells are subsequently screened for expression of the selected gene. The cells expressing these selected genes are then incubated in the presence of a compound of unknown 25 biological activity and further assayed for the ability of the compound to affect the expressed gene product.

Similarly, the attachment enhanced 293 cells of the invention may be used to identify antagonists of the MSR gene, i.e., to develop agents for atherosclerosis. Suitable assays for identifying antagonists to an expressed gene product are well 30 known to those of skill in the art. See, T. Kodama *et al.*, Nature, 343:531-535 (1990), A.M. Pearson *et al.*, J. Biol. Chem., 268:3554 (1993).

The surprising result of enhanced attachment demonstrated by 293 cells transfected with MSR genes is not demonstrated when other cells, such as Chinese Hamster Ovary (CHO) cells, are transfected with MSR I or MSR II. To the inventors' knowledge, no other cell line has demonstrated this result when

5 transfected with MSR genes.

The following examples illustrate the preferred methods for preparing the modified 293 cells of the invention and uses therefor. These examples are illustrative only and are not intended to limit the scope of the invention.

10 Example 1 - Calcium phosphate transfection of macrophage scavenger receptor I and II into human embryonic kidney 293 cells

The macrophage scavenger receptor I or II cDNAs [SEQ ID NO:1 and 3, respectively] were subcloned into the mammalian expression vector pCDN in the correct orientation [N. Aiyar, *Mol. Cell. Biochem.*, 131:75-86 (1994)].

15 The resulting construct containing the macrophage scavenger receptor I or II cDNA was used to transfect human embryonic kidney (HEK) 293 cells by calcium phosphate transfection. One day prior to the transfection, the HEK 293 cells were plated into 10 cm dishes at a density of 2×10^5 cells, so that the cells would be approximately 10% confluent within 24 hours. The cells were seeded into Eagle's

20 Minimal Essential Medium (EMEM) supplemented with 2mM L-glutamine and 10% fetal bovine serum (FBS).

The DNA was prepared for transfection by sterile ethanol precipitation. Following ethanol precipitation, the DNA pellet was dried inside a tissue culture hood. The pellet was then resuspended in 450 μ L of sterile water and 50 μ L of 2.5

25 M CaCl_2 . Ten μ g of DNA were used per 10 cm dish. While gently swirling the DNA mixture, 500 μ L of sterile 2x BBS (50mM N,N-bis 2-hydroxyethyl-2-aminoethane sulfonic acid, 280mM NaCl, and 1.5mM Na_2HPO_4) was added. The BBS/DNA- CaCl_2 solution was allowed to form a precipitate by sitting at room temperature for 10-20 minutes.

30 The solution was then gently mixed to ensure adequate suspension of the precipitate and then added dropwise into the 10 cm dish of cells. The plate was

gently swirled to distribute contents evenly. After a 12-16 hour incubation, the medium was carefully removed, and the cells were washed once with 5 ml of PBS (without Ca^{2+} or Mg^{2+}) followed by the addition of 10ml of EMEM supplemented with 2mM L-glutamine and 10% FBS.

5 Following an overnight incubation, the medium was removed, and the cells were carefully washed once with 5 ml of PBS (without Ca^{2+} or Mg^{2+}). To initiate selection, 10 ml of fresh EMEM with L-glutamine supplemented with 2 mM L-glutamine, 10% FBS and 0.4 mg/ml of geneticin (GIBCO-BRL) were added. Two or three days later, the medium was changed.

10 After approximately 2-3 weeks, each plate was examined under the microscope for small patches of growing cells. The patches were grown large enough to be seen as small spots on the bottom of the plate. Once at this stage, all of the medium was removed and
3 μL of trypsin was added directly to the patch of cells. By pipetting up and down
15 several times, the patch of cells was transferred to a 24 well dish containing 1 ml of medium with geneticin. The cells were expanded from this 24 well stage to a 6 well plate or T-25 Flask. Because the 293 cells grow best in conditioned medium, cells were fed based on their rate of growth, but typically not more than once a week.

20 Example 2 - Comparison of transfected and untransfected 293 cells

To demonstrate the surprising results of the above transfection, and the greater accuracy obtained in using the transfected 293 cells in assays, transfected 293 cells of this invention and untransfected 293 cells were seeded at the same cell density (100,000 per well) into 24-well plastic tissue culture dishes. These cells
25 were allowed to grow for two days before testing. Cell growth appeared to be equivalent.

The same biochemical assay was performed on the transfected and untransfected cells.

The presence of macrophage scavenger receptors was confirmed by
30 incubating transfected 293 cells with ^{125}I -acetylated LDL at a concentration of approximately 5 $\mu\text{g}/\text{ml}$ (specific activity ~100-300 cpm/ng protein) for 5 hours at

37°C, essentially as described in J. Ashkenas *et al.*, J. Lipid Res., 34:983-1000 (1993). In replicate experiments, ¹²⁵[I]-acetylated LDL binding/uptake amounted to an average of 1.75 µg/mg protein (n=76). Where it has been possible to measure ¹²⁵[I]-acetylated LDL binding/uptake to untransfected 293 cells, the average was 0.20 µg/mg protein (n=6). After the assays were performed on the cells, they were dissolved in 0.1 M NaOH, and aliquots were used to determine total protein concentration by the Pierce BCA assay with bovine serum albumin as the standard. In an attempt to keep as many untransfected cells as possible attached to the culture dish, the untransfected cells were washed only twice, while the transfected cells were washed seven times as per the procedure cited above.

Superior attachment of the transfected cells was observed in a comparison of recoverable protein, with an average of 113±2.3 µg protein/well (n=24) versus the untransfected cells with an average of 21.8±4.8 µg protein/well (n=12).

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Attachment Enhanced 293 Cells

(iii) NUMBER OF SEQUENCES: 4

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(F) ZIP: 19406-5090

(v) COMPUTER READABLE FORM:

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(B) COMPUTER: IBM PC compatible

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(viii) ATTORNEY/AGENT INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2028 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 47..1402

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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                                   Met Glu Gln
                                   1

TGG GAT CAC TTT CAC AAT CAA CAG GAG GAC ACT GAT AGC TGC TCC GAA      103
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   5              10              15

TCT GTG AAA TTT GAT GCT CGC TCA ATG ACA GCT TTG CTT CCT CCG AAT      151
Ser Val Lys Phe Asp Ala Arg Ser Met Thr Ala Leu Leu Pro Pro Asn
  20              25              30              35

CCT AAA AAC AGC CCT TCC CTT CAA GAG AAA CTG AAG TCC TTC AAA GCT      199
Pro Lys Asn Ser Pro Ser Leu Gln Glu Lys Leu Lys Ser Phe Lys Ala
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GCA CTG ATT GCC CTT TAC CTC CTC GTG TTT GCA GTT CTC ATC CCT CTC      247
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TCA GTT AGT TCA ACT AAT GCA AAT GAT ATA ACT CAA AGT CTC ACG GGA      343
Ser Val Ser Ser Thr Asn Ala Asn Asp Ile Thr Gln Ser Leu Thr Gly
          85              90              95

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| Glu His Met Ser Asn Met Glu Lys Arg Ile Gln His Ile Leu Asp Met | |
| 120 125 130 | |
| GAA GCC AAC CTC ATG GAC ACA GAG CAT TTC CAA AAT TTC AGC ATG ACA | 487 |
| Glu Ala Asn Leu Met Asp Thr Glu His Phe Gln Asn Phe Ser Met Thr | |
| 135 140 145 | |
| ACT GAT CAA AGA TTT AAT GAC ATT CTT CTG CAG CTA AGT ACC TTG TTT | 535 |
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| 215 220 225 | |
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| Ala Met Lys Glu Glu Gln Val His Leu Glu Gln Glu Ile Lys Gly Glu | |
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| 245 250 255 | |

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| | 325 | | | | | 330 | | | | | 335 | | | | | |
| AGT | GGA | AAC | ACA | TTA | ACT | CCA | TTT | ACG | AAA | GTT | CGA | CTG | GTC | GGT | GGG | 1111 |
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| | | | 375 | | | | | 380 | | | | | 385 | | | |
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| His | Phe | Gly | Gln | Gly | Thr | Gly | Pro | Ile | Trp | Leu | Asn | Glu | Val | Phe | Cys | |
| | 405 | | | | | 410 | | | | | 415 | | | | | |
| TTT | GGG | AGA | GAA | TCA | TCT | ATT | GAA | GAA | TGT | AAA | ATT | CGG | CAA | TGG | GGG | 1351 |
| Phe | Gly | Arg | Glu | Ser | Ser | Ile | Glu | Glu | Cys | Lys | Ile | Arg | Gln | Trp | Gly | |
| 420 | | | | | 425 | | | | 430 | | | | | 435 | | |

ACA AGA GCC TGT TCA CAT TCT GAA GAT GCT GGA GTC ACT TGC ACT TTA 1399
 Thr Arg Ala Cys Ser His Ser Glu Asp Ala Gly Val Thr Cys Thr Leu
 440 445 450

TAA TGCATCATAT TTTCATTAC AACTATGAAA TCGCTGCTCA AAAATGATTT 1452
 *

TATTACCTTG TTCCTGTAAA ATCCATTAA TCAATATTTA AGAGATTAAG AATATTGCCC 1512

AAATAATATT TTAGATTACA GGATTAATAT ATTGAACACC TTCATGCTTA CTATTTTATG 1572

TCTATATTTA AATCATTTTA ACTTCTATAG GTTTTTAAAT GGAATTTTCT AATATAATGA 1632

CTTATATGCT GAATTGAACA TTTTGAAGTT TATAGCTTCC AGATTACAAA GGCCAAGGGT 1692

AATAGAAATG CATACCAGTA ATTGGCTCCA ATTCATAATA TGTTACCAG GAGATTACAA 1752

TTTTTTGCTC TTCTTGCTT TGTAATCTAT TTAGTTGATT TTAATTACTT TCTGAATAAC 1812

GGAAGGGATC AGAAGATATC TTTTGTGCCT AGATTGCAAA ATCTCCAATC CACACATATT 1872

GTTTTAAAT AAGAATGTTA TCCAATATT AAGATATCTC AATGTGCAAT AACTTGTGTA 1932

TTAGATATCA ATGTTAATGA TATGTCTTGG CCACTATGGA CCAGGGAGCT TATTTTCTT 1992

GTCATGTACT GACAACTGTT TAATTGAATC ATGAAG 2028

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 452 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Gln Trp Asp His Phe His Asn Gln Gln Glu Asp Thr Asp Ser
 1 5 10 15

Cys Ser Glu Ser Val Lys Phe Asp Ala Arg Ser Met Thr Ala Leu Leu
 20 25 30

Pro Pro Asn Pro Lys Asn Ser Pro Ser Leu Gln Glu Lys Leu Lys Ser
 35 40 45

Phe Lys Ala Ala Leu Ile Ala Leu Tyr Leu Leu Val Phe Ala Val Leu
 50 55 60

Ile Pro Leu Ile Gly Ile Val Ala Ala Gln Leu Leu Lys Trp Glu Thr
 65 70 75 80

Lys Asn Cys Ser Val Ser Ser Thr Asn Ala Asn Asp Ile Thr Gln Ser
 85 90 95

Leu Thr Gly Lys Gly Asn Asp Ser Glu Glu Glu Met Arg Phe Gln Glu
 100 105 110

Val Phe Met Glu His Met Ser Asn Met Glu Lys Arg Ile Gln His Ile
 115 120 125

Leu Asp Met Glu Ala Asn Leu Met Asp Thr Glu His Phe Gln Asn Phe
 130 135 140

Ser Met Thr Thr Asp Gln Arg Phe Asn Asp Ile Leu Leu Gln Leu Ser
 145 150 155 160

Thr Leu Phe Ser Ser Val Gln Gly His Gly Asn Ala Ile Asp Glu Ile
 165 170 175

Ser Lys Ser Leu Ile Ser Leu Asn Thr Thr Leu Leu Asp Leu Gln Leu
 180 185 190

Asn Ile Glu Asn Leu Asn Gly Lys Ile Gln Glu Asn Thr Phe Lys Gln
 195 200 205

Gln Glu Glu Ile Ser Lys Leu Glu Glu Arg Val Tyr Asn Val Ser Ala
 210 215 220

Glu Ile Met Ala Met Lys Glu Glu Gln Val His Leu Glu Gln Glu Ile
 225 230 235 240

Lys Gly Glu Val Lys Val Leu Asn Asn Ile Thr Asn Asp Leu Arg Leu
 245 250 255
 Lys Asp Trp Glu His Ser Gln Thr Leu Arg Asn Ile Thr Leu Ile Gln
 260 265 270
 Gly Pro Pro Gly Pro Pro Gly Glu Lys Gly Asp Arg Gly Pro Thr Gly
 275 280 285
 Glu Ser Gly Pro Arg Gly Phe Pro Gly Pro Ile Gly Pro Pro Gly Leu
 290 295 300
 Lys Gly Asp Arg Gly Ala Ile Gly Phe Pro Gly Ser Arg Gly Leu Pro
 305 310 315 320
 Gly Tyr Ala Gly Arg Pro Gly Asn Ser Gly Pro Lys Gly Gln Lys Gly
 325 330 335
 Glu Lys Gly Ser Gly Asn Thr Leu Thr Pro Phe Thr Lys Val Arg Leu
 340 345 350
 Val Gly Gly Ser Gly Pro His Glu Gly Arg Val Glu Ile Leu His Ser
 355 360 365
 Gly Gln Trp Gly Thr Ile Cys Asp Asp Arg Trp Glu Val Arg Val Gly
 370 375 380
 Gln Val Val Cys Arg Ser Leu Gly Tyr Pro Gly Val Gln Ala Val His
 385 390 395 400
 Lys Ala Ala His Phe Gly Gln Gly Thr Gly Pro Ile Trp Leu Asn Glu
 405 410 415
 Val Phe Cys Phe Gly Arg Glu Ser Ser Ile Glu Glu Cys Lys Ile Arg
 420 425 430
 Gln Trp Gly Thr Arg Ala Cys Ser His Ser Glu Asp Ala Gly Val Thr
 435 440 445
 Cys Thr Leu *
 450

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1367 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 67..1143

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | |
|---|-----|
| TAGGTTTCAA TTGTAAAGAG AGAGAAGTGG ATAAATCAGT GCTGCTTTCT TTAGGACGAA | 60 |
| AGAAGT ATG GAG CAG TGG GAT CAC TTT CAC AAT CAA CAG GAG GAC ACT | 108 |
| Met Glu Gln Trp Asp His Phe His Asn Gln Gln Glu Asp Thr | |
| 1 5 10 | |
| GAT AGC TGC TCC GAA TCT GTG AAA TTT GAT GCT CGC TCA ATG ACA GCT | 156 |
| Asp Ser Cys Ser Glu Ser Val Lys Phe Asp Ala Arg Ser Met Thr Ala | |
| 15 20 25 30 | |
| TTG CTT CCT CCG AAT CCT AAA AAC AGC CCT TCC CTT CAA GAG AAA CTG | 204 |
| Leu Leu Pro Pro Asn Pro Lys Asn Ser Pro Ser Leu Gln Glu Lys Leu | |
| 35 40 45 | |
| AAG TCC TTC AAA GCT GCA CTG ATT GCC CTT TAC CTC CTC GTG TTT GCA | 252 |
| Lys Ser Phe Lys Ala Ala Leu Ile Ala Leu Tyr Leu Leu Val Phe Ala | |
| 50 55 60 | |
| GTT CTC ATC CCT CTC ATT GGA ATA GTG GCA GCT CAA CTC CTG AAG TGG | 300 |
| Val Leu Ile Pro Leu Ile Gly Ile Val Ala Ala Gln Leu Leu Lys Trp | |
| 65 70 75 | |
| GAA ACG AAG AAT TGC TCA GTT AGT TCA ACT AAT GCA AAT GAT ATA ACT | 348 |
| Glu Thr Lys Asn Cys Ser Val Ser Ser Thr Asn Ala Asn Asp Ile Thr | |
| 80 85 90 | |

| | |
|---|-----|
| CAA AGT CTC ACG GGA AAA GGA AAT GAC AGC GAA GAG GAA ATG AGA TTT | 396 |
| Gln Ser Leu Thr Gly Lys Gly Asn Asp Ser Glu Glu Glu Met Arg Phe | |
| 95 100 105 110 | |
| CAA GAA GTC TTT ATG GAA CAC ATG AGC AAC ATG GAG AAG AGA ATC CAG | 444 |
| Gln Glu Val Phe Met Glu His Met Ser Asn Met Glu Lys Arg Ile Gln | |
| 115 120 125 | |
| CAT ATT TTA GAC ATG GAA GCC AAC CTC ATG GAC ACA GAG CAT TTC CAA | 492 |
| His Ile Leu Asp Met Glu Ala Asn Leu Met Asp Thr Glu His Phe Gln | |
| 130 135 140 | |
| AAT TTC AGC ATG ACA ACT GAT CAA AGA TTT AAT GAC ATT CTT CTG CAG | 540 |
| Asn Phe Ser Met Thr Thr Asp Gln Arg Phe Asn Asp Ile Leu Leu Gln | |
| 145 150 155 | |
| CTA AGT ACC TTG TTT TCC TCA GTC CAG GGA CAT GGG AAT GCA ATA GAT | 588 |
| Leu Ser Thr Leu Phe Ser Ser Val Gln Gly His Gly Asn Ala Ile Asp | |
| 160 165 170 | |
| GAA ATC TCC AAG TCC TTA ATA AGT TTG AAT ACC ACA TTG CTT GAT TTG | 636 |
| Glu Ile Ser Lys Ser Leu Ile Ser Leu Asn Thr Thr Leu Leu Asp Leu | |
| 175 180 185 190 | |
| CAG CTC AAC ATA GAA AAT CTG AAT GGC AAA ATC CAA GAG AAT ACC TTC | 684 |
| Gln Leu Asn Ile Glu Asn Leu Asn Gly Lys Ile Gln Glu Asn Thr Phe | |
| 195 200 205 | |
| AAA CAA CAA GAG GAA ATC AGT AAA TTA GAG GAG CGT GTT TAC AAT GTA | 732 |
| Lys Gln Gln Glu Glu Ile Ser Lys Leu Glu Glu Arg Val Tyr Asn Val | |
| 210 215 220 | |
| TCA GCA GAA ATT ATG GCT ATG AAA GAA GAA CAA GTG CAT TTG GAA CAG | 780 |
| Ser Ala Glu Ile Met Ala Met Lys Glu Glu Gln Val His Leu Glu Gln | |
| 225 230 235 | |
| GAA ATA AAA GGA GAA GTG AAA GTA CTG AAT AAC ATC ACT AAT GAT CTC | 828 |
| Glu Ile Lys Gly Glu Val Lys Val Leu Asn Asn Ile Thr Asn Asp Leu | |
| 240 245 250 | |
| AGA CTG AAA GAT TGG GAA CAT TCT CAG ACC TTG AGA AAT ATC ACT TTA | 876 |
| Arg Leu Lys Asp Trp Glu His Ser Gln Thr Leu Arg Asn Ile Thr Leu | |
| 255 260 265 270 | |

| | |
|---|------|
| ATT CAA GGT CCT CCT GGA CCC CCG GGT GAA AAA GGA GAT CGA GGT CCC Ile Gln Gly Pro Pro Gly Pro Pro Gly Glu Lys Gly Asp Arg Gly Pro 275 280 285 | 924 |
| ACT GGA GAA AGT GGT CCA CGA GGA TTT CCA GGT CCA ATA GGT CCT CCG Thr Gly Glu Ser Gly Pro Arg Gly Phe Pro Gly Pro Ile Gly Pro Pro 290 295 300 | 972 |
| GGT CTT AAA GGT GAT CGG GGA GCA ATT GGC TTT CCT GGA AGT CGA GGA Gly Leu Lys Gly Asp Arg Gly Ala Ile Gly Phe Pro Gly Ser Arg Gly 305 310 315 | 1020 |
| CTC CCA GGA TAT GCC GGA AGG CCA GGA AAT TCT GGA CCA AAA GGC CAG Leu Pro Gly Tyr Ala Gly Arg Pro Gly Asn Ser Gly Pro Lys Gly Gln 320 325 330 | 1068 |
| AAA GGG GAA AAG GGG AGT GGA AAC ACA TTA AGA CCA GTA CAA CTC ACT Lys Gly Glu Lys Gly Ser Gly Asn Thr Leu Arg Pro Val Gln Leu Thr 335 340 345 350 | 1116 |
| GAT CAT ATT AGG GCA GGG CCC TCT TAA GATCAGGTGG GTTGGGCGGG Asp His Ile Arg Ala Gly Pro Ser * | 1163 |
| 355 | |
| ACATCCTCTG CTACCATCTC ATTAAAAGGC CCTTCACCTC TGGACAAGTC ATCTGCAACA | 1223 |
| ACTGACTTCC AAGATCCTTT TGTGACTCCT CCAAATGACT TTGGTTCCCG TGTGTACCT | 1283 |
| GACTTCCACA TGGCCTTCTC TCCTGGTCCC TGGTGCTGTT TGGGCCTCTG CTCCCATGCT | 1343 |
| CATACCTCTT CTTACTCCAA TTAC | 1367 |

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Glu | Gln | Trp | Asp | His | Phe | His | Asn | Gln | Gln | Glu | Asp | Thr | Asp | Ser | 1 | 5 | 10 | 15 |
| Cys | Ser | Glu | Ser | Val | Lys | Phe | Asp | Ala | Arg | Ser | Met | Thr | Ala | Leu | Leu | 20 | 25 | 30 | |
| Pro | Pro | Asn | Pro | Lys | Asn | Ser | Pro | Ser | Leu | Gln | Glu | Lys | Leu | Lys | Ser | 35 | 40 | 45 | |
| Phe | Lys | Ala | Ala | Leu | Ile | Ala | Leu | Tyr | Leu | Leu | Val | Phe | Ala | Val | Leu | 50 | 55 | 60 | |
| Ile | Pro | Leu | Ile | Gly | Ile | Val | Ala | Ala | Gln | Leu | Leu | Lys | Trp | Glu | Thr | 65 | 70 | 75 | 80 |
| Lys | Asn | Cys | Ser | Val | Ser | Ser | Thr | Asn | Ala | Asn | Asp | Ile | Thr | Gln | Ser | 85 | 90 | 95 | |
| Leu | Thr | Gly | Lys | Gly | Asn | Asp | Ser | Glu | Glu | Glu | Met | Arg | Phe | Gln | Glu | 100 | 105 | 110 | |
| Val | Phe | Met | Glu | His | Met | Ser | Asn | Met | Glu | Lys | Arg | Ile | Gln | His | Ile | 115 | 120 | 125 | |
| Leu | Asp | Met | Glu | Ala | Asn | Leu | Met | Asp | Thr | Glu | His | Phe | Gln | Asn | Phe | 130 | 135 | 140 | |
| Ser | Met | Thr | Thr | Asp | Gln | Arg | Phe | Asn | Asp | Ile | Leu | Leu | Gln | Leu | Ser | 145 | 150 | 155 | 160 |
| Thr | Leu | Phe | Ser | Ser | Val | Gln | Gly | His | Gly | Asn | Ala | Ile | Asp | Glu | Ile | 165 | 170 | 175 | |
| Ser | Lys | Ser | Leu | Ile | Ser | Leu | Asn | Thr | Thr | Leu | Leu | Asp | Leu | Gln | Leu | 180 | 185 | 190 | |
| Asn | Ile | Glu | Asn | Leu | Asn | Gly | Lys | Ile | Gln | Glu | Asn | Thr | Phe | Lys | Gln | 195 | 200 | 205 | |
| Gln | Glu | Glu | Ile | Ser | Lys | Leu | Glu | Glu | Arg | Val | Tyr | Asn | Val | Ser | Ala | 210 | 215 | 220 | |

Glu Ile Met Ala Met Lys Glu Glu Gln Val His Leu Glu Gln Glu Ile
 225 230 235 240

Lys Gly Glu Val Lys Val Leu Asn Asn Ile Thr Asn Asp Leu Arg Leu
 245 250 255

Lys Asp Trp Glu His Ser Gln Thr Leu Arg Asn Ile Thr Leu Ile Gln
 260 265 270

Gly Pro Pro Gly Pro Pro Gly Glu Lys Gly Asp Arg Gly Pro Thr Gly
 275 280 285

Glu Ser Gly Pro Arg Gly Phe Pro Gly Pro Ile Gly Pro Pro Gly Leu
 290 295 300

Lys Gly Asp Arg Gly Ala Ile Gly Phe Pro Gly Ser Arg Gly Leu Pro
 305 310 315 320

Gly Tyr Ala Gly Arg Pro Gly Asn Ser Gly Pro Lys Gly Gln Lys Gly
 325 330 335

Glu Lys Gly Ser Gly Asn Thr Leu Arg Pro Val Gln Leu Thr Asp His
 340 345 350

Ile Arg Ala Gly Pro Ser *
 355

WHAT IS CLAIMED IS:

1. Human embryonic kidney 293 cells transfected with a mammalian scavenger receptor gene, said cells demonstrating an enhanced ability to attach to a solid support.
2. The cells according to claim 1 wherein said receptor gene is a human macrophage scavenger receptor gene Type I.
3. The cells according to claim 1 wherein the receptor gene is characterized by the sequence of GenBank accession number D90187.
4. The cells according to claim 1 wherein said receptor gene is a human macrophage scavenger receptor gene Type II.
5. The cells according to claim 1 wherein the receptor gene is characterized by the sequence of GenBank accession number D90188.
6. The cells according to claim 1 wherein said receptor gene is a macrophage scavenger receptor gene of a non-human species.
7. A solid support to which is attached human embryonic kidney 293 cells transfected with a mammalian scavenger receptor gene.
8. The support according to claim 7 wherein said receptor gene is a human macrophage scavenger receptor gene Type I.
9. The support according to claim 7 wherein the receptor gene is characterized by the sequence of GenBank accession number D90187.
10. The support according to claim 7 wherein said receptor gene is a human macrophage scavenger receptor gene Type II.
11. The support according to claim 7 wherein the receptor gene is characterized by the sequence of GenBank accession number D90188.
12. The support according to claim 7 wherein said receptor gene is a macrophage scavenger receptor gene of a non-human species.
13. A method of enhancing the ability of human embryonic kidney cells to attach to a solid support comprising the steps of:
 - (a) providing cells from a 293 cell line; and

(b) transfecting the cells with a mammalian scavenger receptor gene;

wherein the transfected cells are characterized by an enhanced ability to attach to said solid support.

5 14. The method according to claim 13 further comprising transfecting said cells with a second selected gene.

 15. The method according to claim 14 wherein the selected gene is a selection marker.

 16. The method according to claim 15 wherein the gene is a selectable
10 resistance marker.

 17. A method of screening a compounds for biological activity comprising the steps of:

 (a) providing on a solid support human embryonic kidney 293
cells co-transfected with a mammalian scavenger receptor gene and a second
15 selected gene which encodes a protein having a biological activity;

 (b) measuring expression of the protein encoded by said second selected gene;

 (c) incubating said co-transfected 293 cells in the presence of a compound of unknown biological activity;

20 (d) screening the cells of (c) for the ability of the compound to alter said biological activity.

 18. The method according to claim 17 wherein the receptor gene is a human macrophage scavenger receptor gene Type I or Type II.

 19. An improved method for screening a compound for biological
25 activity comprising measuring in a transfected cell the expression of a protein encoded by a selected gene; incubating said transfected cell in the presence of a compound of unknown biological activity; and screening the cell for the ability of said compound to alter said biological activity, the improvement comprising
employing as said transfected cell, human embryonic kidney 293 cells co-transfected
30 with a mammalian scavenger receptor gene and said selected gene, said cells attached to a solid support.

20. An improved method for performing a biological assay on a cell attached to a solid support, wherein said assay involves at least one washing step, said improvement comprising employing as said attached cell, human embryonic kidney 293 cells co-transfected with a mammalian scavenger receptor gene.
- 5 21. An improved method for measuring the production of a protein in a cell attached to a solid support, said improvement comprising employing as said attached cell, human embryonic kidney 293 cells co-transfected with a mammalian scavenger receptor gene.

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Human Macrophage Scavenger Receptor Type I

Nucleic acid SEQ ID NO:1 and Amino Acid SEQ ID NO:2 Sequences

| | |
|---|-----|
| AGAGAAGTGG ATAAATCAGT GCTGCTTTCT TTAGGACGAA AGAAGT ATG GAG CAG | 55 |
| Met Glu Gln | |
| 1 | |
| TGG GAT CAC TTT CAC AAT CAA CAG GAG GAC ACT GAT AGC TGC TCC GAA | 103 |
| Trp Asp His Phe His Asn Gln Gln Glu Asp Thr Asp Ser Cys Ser Glu | |
| 5 10 15 | |
| TCT GTG AAA TTT GAT GCT CGC TCA ATG ACA GCT TTG CTT CCT CCG AAT | 151 |
| Ser Val Lys Phe Asp Ala Arg Ser Met Thr Ala Leu Leu Pro Pro Asn | |
| 20 25 30 35 | |
| CCT AAA AAC AGC CCT TCC CTT CAA GAG AAA CTG AAG TCC TTC AAA GCT | 199 |
| Pro Lys Asn Ser Pro Ser Leu Gln Glu Lys Leu Lys Ser Phe Lys Ala | |
| 40 45 50 | |
| GCA CTG ATT GCC CTT TAC CTC CTC GTG TTT GCA GTT CTC ATC CCT CTC | 247 |
| Ala Leu Ile Ala Leu Tyr Leu Leu Val Phe Ala Val Leu Ile Pro Leu | |
| 55 60 65 | |
| ATT GGA ATA GTG GCA GCT CAA CTC CTG AAG TGG GAA ACG AAG AAT TGC | 295 |
| Ile Gly Ile Val Ala Ala Gln Leu Leu Lys Trp Glu Thr Lys Asn Cys | |
| 70 75 80 | |
| TCA GTT AGT TCA ACT AAT GCA AAT GAT ATA ACT CAA AGT CTC ACG GGA | 343 |
| Ser Val Ser Ser Thr Asn Ala Asn Asp Ile Thr Gln Ser Leu Thr Gly | |
| 85 90 95 | |
| AAA GGA AAT GAC AGC GAA GAG GAA ATG AGA TTT CAA GAA GTC TTT ATG | 391 |
| Lys Gly Asn Asp Ser Glu Glu Glu Met Arg Phe Gln Glu Val Phe Met | |
| 100 105 110 115 | |
| GAA CAC ATG AGC AAC ATG GAG AAG AGA ATC CAG CAT ATT TTA GAC ATG | 439 |
| Glu His Met Ser Asn Met Glu Lys Arg Ile Gln His Ile Leu Asp Met | |
| 120 125 130 | |
| GAA GCC AAC CTC ATG GAC ACA GAG CAT TTC CAA AAT TTC AGC ATG ACA | 487 |
| Glu Ala Asn Leu Met Asp Thr Glu His Phe Gln Asn Phe Ser Met Thr | |
| 135 140 145 | |
| ACT GAT CAA AGA TTT AAT GAC ATT CTT CTG CAG CTA AGT ACC TTG TTT | 535 |
| Thr Asp Gln Arg Phe Asn Asp Ile Leu Leu Gln Leu Ser Thr Leu Phe | |
| 150 155 160 | |
| TCC TCA GTC CAG GGA CAT GGG AAT GCA ATA GAT GAA ATC TCC AAG TCC | 583 |
| Ser Ser Val Gln Gly His Gly Asn Ala Ile Asp Glu Ile Ser Lys Ser | |
| 165 170 175 | |
| TTA ATA AGT TTG AAT ACC ACA TTG CTT GAT TTG CAG CTC AAC ATA GAA | 631 |
| Leu Ile Ser Leu Asn Thr Thr Leu Leu Asp Leu Gln Leu Asn Ile Glu | |
| 180 185 190 195 | |
| AAT CTG AAT GGC AAA ATC CAA GAG AAT ACC TTC AAA CAA CAA GAG GAA | 679 |
| Asn Leu Asn Gly Lys Ile Gln Glu Asn Thr Phe Lys Gln Gln Glu Glu | |
| 200 205 210 | |

Fig. 1A

SUBSTITUTE SHEET (RULE 26)

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| | |
|---|------|
| ATC AGT AAA TTA GAG GAG CGT GTT TAC AAT GTA TCA GCA GAA ATT ATG Ile Ser Lys Leu Glu Glu Arg Val Tyr Asn Val Ser Ala Glu Ile Met 215 220 225 | 727 |
| GCT ATG AAA GAA GAA CAA GTG CAT TTG GAA CAG GAA ATA AAA GGA GAA Ala Met Lys Glu Glu Gln Val His Leu Glu Gln Glu Ile Lys Gly Glu 230 235 240 | 775 |
| GTG AAA GTA CTG AAT AAC ATC ACT AAT GAT CTC AGA CTG AAA GAT TGG Val Lys Val Leu Asn Asn Ile Thr Asn Asp Leu Arg Leu Lys Asp Trp 245 250 255 | 823 |
| GAA CAT TCT CAG ACC TTG AGA AAT ATC ACT TTA ATT CAA GGT CCT CCT Glu His Ser Gln Thr Leu Arg Asn Ile Thr Leu Ile Gln Gly Pro Pro 260 265 270 275 | 871 |
| GGA CCC CCG GGT GAA AAA GGA GAT CGA GGT CCC ACT GGA GAA AGT GGT Gly Pro Pro Gly Glu Lys Gly Asp Arg Gly Pro Thr Gly Glu Ser Gly 280 285 290 | 919 |
| CCA CGA GGA TTT CCA GGT CCA ATA GGT CCT CCG GGT CTT AAA GGT GAT Pro Arg Gly Phe Pro Gly Pro Ile Gly Pro Pro Gly Leu Lys Gly Asp 295 300 305 | 967 |
| CGG GGA GCA ATT GGC TTT CCT GGA AGT CGA GGA CTC CCA GGA TAT GCC Arg Gly Ala Ile Gly Phe Pro Gly Ser Arg Gly Leu Pro Gly Tyr Ala 310 315 320 | 1015 |
| GGA AGG CCA GGA AAT TCT GGA CCA AAA GGC CAG AAA GGG GAA AAG GGG Gly Arg Pro Gly Asn Ser Gly Pro Lys Gly Gln Lys Gly Glu Lys Gly 325 330 335 | 1063 |
| AGT GGA AAC ACA TTA ACT CCA TTT ACG AAA GTT CGA CTG GTC GGT GGG Ser Gly Asn Thr Leu Thr Pro Phe Thr Lys Val Arg Leu Val Gly Gly 340 345 350 355 | 1111 |
| AGC GGC CCT CAC GAG GGG AGA GTG GAG ATA CTC CAC AGC GGC CAG TGG Ser Gly Pro His Glu Gly Arg Val Glu Ile Leu His Ser Gly Gln Trp 360 365 370 | 1159 |
| GGT ACA ATT TGT GAC GAT CGC TGG GAA GTG CGC GTT GGA CAG GTC GTC Gly Thr Ile Cys Asp Asp Arg Trp Glu Val Arg Val Gly Gln Val Val 375 380 385 | 1207 |
| TGT AGG AGC TTG GGA TAC CCA GGT GTT CAA GCC GTG CAC AAG GCA GCT Cys Arg Ser Leu Gly Tyr Pro Gly Val Gln Ala Val His Lys Ala Ala 390 395 400 | 1255 |
| CAC TTT GGA CAA GGT ACT GGT CCA ATA TGG CTG AAT GAA GTG TTT TGT His Phe Gly Gln Gly Thr Gly Pro Ile Trp Leu Asn Glu Val Phe Cys 405 410 415 | 1303 |
| TTT GGG AGA GAA TCA TCT ATT GAA GAA TGT AAA ATT CGG CAA TGG GGG Phe Gly Arg Glu Ser Ser Ile Glu Glu Cys Lys Ile Arg Gln Trp Gly 420 425 430 435 | 1351 |
| ACA AGA GCC TGT TCA CAT TCT GAA GAT GCT GGA GTC ACT TGC ACT TTA Thr Arg Ala Cys Ser His Ser Glu Asp Ala Gly Val Thr Cys Thr Leu 440 445 450 | 1399 |

Fig. 1B

SUBSTITUTE SHEET (RULE 26)

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| | |
|---|------|
| TAA TGCATCATAT TTTCATTAC AACTATGAAA TCGCTGCTCA AAAATGATTT | 1452 |
| * | |
| TATTACCTTG TTCCTGTAAA ATCCATTTAA TCAATATTTA AGAGATTAAG AATATTGCCC | 1512 |
| AAATAATATT TTAGATTACA GGATTAATAT ATTGAACACC TTCATGCTTA CTATTTTATG | 1572 |
| TCTATATTTA AATCATTTTA ACTTCTATAG GTTTTTAAAT GGAATTTTCT AATATAATGA | 1632 |
| CTTATATGCT GAATTGAACA TTTTGAAGTT TATAGCTTCC AGATTACAAA GGCCAAGGGT | 1692 |
| AATAGAAATG CATACCAGTA ATTGGCTCCA ATTCATAATA TGTTACCAG GAGATTACAA | 1752 |
| TTTTTTGCTC TTCTTGCTT TGTAATCTAT TTAGTTGATT TTAATTACTT TCTGAATAAC | 1812 |
| GGAAGGGATC AGAAGATATC TTTTGTGCCT AGATTGCAAA ATCTCCAATC CACACATATT | 1872 |
| GTTTTAAAAT AAGAATGTTA TCCAACATT AAGATATCTC AATGTGCAAT AACTTGTGTA | 1932 |
| TTAGATATCA ATGTTAATGA TATGTCTTGG CCACTATGGA CCAGGGAGCT TATTTTCTT | 1992 |
| GTCATGTACT GACAACTGTT TAATTGAATC ATGAAG | 2028 |

Fig. 1C

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Human Macrophage Scavenger Receptor Type II

Nucleic acid SEQ ID NO:3 and Amino Acid SEQ ID NO:4 Sequences

| | |
|---|-----|
| TAGGTTTCAA TTGTAAAGAG AGAGAAGTGG ATAAATCAGT GCTGCTTTCT TTAGGACGAA | 60 |
| AGAAGT ATG GAG CAG TGG GAT CAC TTT CAC AAT CAA CAG GAG GAC ACT | 108 |
| Met Glu Gln Trp Asp His Phe His Asn Gln Gln Glu Asp Thr | |
| 1 5 10 | |
| GAT AGC TGC TCC GAA TCT GTG AAA TTT GAT GCT CGC TCA ATG ACA GCT | 156 |
| Asp Ser Cys Ser Glu Ser Val Lys Phe Asp Ala Arg Ser Met Thr Ala | |
| 15 20 25 30 | |
| TTG CTT CCT CCG AAT CCT AAA AAC AGC CCT TCC CTT CAA GAG AAA CTG | 204 |
| Leu Leu Pro Pro Asn Pro Lys Asn Ser Pro Ser Leu Gln Glu Lys Leu | |
| 35 40 45 | |
| AAG TCC TTC AAA GCT GCA CTG ATT GCC CTT TAC CTC CTC GTG TTT GCA | 252 |
| Lys Ser Phe Lys Ala Ala Leu Ile Ala Leu Tyr Leu Leu Val Phe Ala | |
| 50 55 60 | |
| GTT CTC ATC CCT CTC ATT GGA ATA GTG GCA GCT CAA CTC CTG AAG TGG | 300 |
| Val Leu Ile Pro Leu Ile Gly Ile Val Ala Ala Gln Leu Leu Lys Trp | |
| 65 70 75 | |
| GAA ACG AAG AAT TGC TCA GTT AGT TCA ACT AAT GCA AAT GAT ATA ACT | 348 |
| Glu Thr Lys Asn Cys Ser Val Ser Ser Thr Asn Ala Asn Asp Ile Thr | |
| 80 85 90 | |
| CAA AGT CTC ACG GGA AAA GGA AAT GAC AGC GAA GAG GAA ATG AGA TTT | 396 |
| Gln Ser Leu Thr Gly Lys Gly Asn Asp Ser Glu Glu Glu Met Arg Phe | |
| 95 100 105 110 | |
| CAA GAA GTC TTT ATG GAA CAC ATG AGC AAC ATG GAG AAG AGA ATC CAG | 444 |
| Gln Glu Val Phe Met Glu His Met Ser Asn Met Glu Lys Arg Ile Gln | |
| 115 120 125 | |
| CAT ATT TTA GAC ATG GAA GCC AAC CTC ATG GAC ACA GAG CAT TTC CAA | 492 |
| His Ile Leu Asp Met Glu Ala Asn Leu Met Asp Thr Glu His Phe Gln | |
| 130 135 140 | |
| AAT TTC AGC ATG ACA ACT GAT CAA AGA TTT AAT GAC ATT CTT CTG CAG | 540 |
| Asn Phe Ser Met Thr Thr Asp Gln Arg Phe Asn Asp Ile Leu Leu Gln | |
| 145 150 155 | |
| CTA AGT ACC TTG TTT TCC TCA GTC CAG GGA CAT GGG AAT GCA ATA GAT | 588 |
| Leu Ser Thr Leu Phe Ser Ser Val Gln Gly His Gly Asn Ala Ile Asp | |
| 160 165 170 | |
| GAA ATC TCC AAG TCC TTA ATA AGT TTG AAT ACC ACA TTG CTT GAT TTG | 636 |
| Glu Ile Ser Lys Ser Leu Ile Ser Leu Asn Thr Thr Leu Leu Asp Leu | |
| 175 180 185 190 | |
| CAG CTC AAC ATA GAA AAT CTG AAT GGC AAA ATC CAA GAG AAT ACC TTC | 684 |
| Gln Leu Asn Ile Glu Asn Leu Asn Gly Lys Ile Gln Glu Asn Thr Phe | |
| 195 200 205 | |

Fig. 2A

5/5

| | |
|---|------|
| AAA CAA CAA GAG GAA ATC AGT AAA TTA GAG GAG CGT GTT TAC AAT GTA Lys Gln Gln Glu Glu Ile Ser Lys Leu Glu Glu Arg Val Tyr Asn Val 210 215 220 | 732 |
| TCA GCA GAA ATT ATG GCT ATG AAA GAA GAA CAA GTG CAT TTG GAA CAG Ser Ala Glu Ile Met Ala Met Lys Glu Glu Gln Val His Leu Glu Gln 225 230 235 | 780 |
| GAA ATA AAA GGA GAA GTG AAA GTA CTG AAT AAC ATC ACT AAT GAT CTC Glu Ile Lys Gly Glu Val Lys Val Leu Asn Asn Ile Thr Asn Asp Leu 240 245 250 | 828 |
| AGA CTG AAA GAT TGG GAA CAT TCT CAG ACC TTG AGA AAT ATC ACT TTA Arg Leu Lys Asp Trp Glu His Ser Gln Thr Leu Arg Asn Ile Thr Leu 255 260 265 270 | 876 |
| ATT CAA GGT CCT CCT GGA CCC CCG GGT GAA AAA GGA GAT CGA GGT CCC Ile Gln Gly Pro Pro Gly Pro Pro Gly Glu Lys Gly Asp Arg Gly Pro 275 280 285 | 924 |
| ACT GGA GAA AGT GGT CCA CGA GGA TTT CCA GGT CCA ATA GGT CCT CCG Thr Gly Glu Ser Gly Pro Arg Gly Phe Pro Gly Pro Ile Gly Pro Pro 290 295 300 | 972 |
| GGT CTT AAA GGT GAT CGG GGA GCA ATT GGC TTT CCT GGA AGT CGA GGA Gly Leu Lys Gly Asp Arg Gly Ala Ile Gly Phe Pro Gly Ser Arg Gly 305 310 315 | 1020 |
| CTC CCA GGA TAT GCC GGA AGG CCA GGA AAT TCT GGA CCA AAA GGC CAG Leu Pro Gly Tyr Ala Gly Arg Pro Gly Asn Ser Gly Pro Lys Gly Gln 320 325 330 | 1068 |
| AAA GGG GAA AAG GGG AGT GGA AAC ACA TTA AGA CCA GTA CAA CTC ACT Lys Gly Glu Lys Gly Ser Gly Asn Thr Leu Arg Pro Val Gln Leu Thr 335 340 345 350 | 1116 |
| GAT CAT ATT AGG GCA GGG CCC TCT TAA GATCAGGTGG GTTGGGCGGG Asp His Ile Arg Ala Gly Pro Ser * | 1163 |
| ACATCCTCTG CTACCATCTC ATTAAAAGGC CCTTCACCTC TGGACAAGTC ATCTGCAACA | 1223 |
| ACTGACTTCC AAGATCCTTT TGTGACTCCT CCAATGACT TTGGTTCCCG TGTTGTACCT | 1283 |
| GACTTCCACA TGGCCTTCTC TCCTGGTCCC TGGTGCTGTT TGGGCCTCTG CTCCCATGCT | 1343 |
| CATACCTCTT CTTACTCCAA TTAC | 1367 |

Fig. 2B

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/08081

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.21, 69.1, 240.1, 240.2, 240.23, 320.1; 530/300, 350; 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| Y | MATSUMOTO et al. Human macrophage scavenger receptors: Primary structure, expression, and localization in atherosclerotic lesions. Proc. Natl. Acad. Sci. December 1990, Vol. 87, pages 9133-9137, especially pages 9133-9136. | 1-21 |
| Y | SPRENGEL et al. Molecular Cloning and Expression of cDNA Encoding a Peripheral-type Benzodiazepine Receptor. Journal of Biological Chemistry. 05 December 1989, Vol. 264, No. 34 pages 20415-20421, especially page 20417. | 1-21 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" earlier document published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

16 AUGUST 1996

Date of mailing of the international search report

04 OCT 1996

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08081

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| Y | KODAMA et al. Type I macrophage scavenger receptor contains α -helical and collagen-like coiled coils. Nature. 08 February 1990, Vol. 343, pages 531-535, especially 531-534. | 1-21 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08081

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

G01N 33/53, 33/567; C12N 1/20, 5/00, 15/00; C07K 1/00, 21/04; A61K 38/00; C12P 21/06

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/7.1, 7.21, 69.1, 240.1, 240.2, 240.23, 320.1; 530/300, 350; 536/23.1, 23.5

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

CAPLUS, MEDLINE, BIOSIS, EMBASE, CONFSCI, DISSABS

search terms: 293 cells, cho cells, human macrophage scavenger receptor, human, msr